

EPA/600/R-03/047
September 2002



Animal Source Identification Using A *Cryptosporidium* DNA Characterization Technique

by

Michael Royer
U.S. Environmental Protection Agency
Edison, New Jersey 08837

Lihua Xiao and Altaf Lal
Centers for Disease Control and Prevention
Atlanta, Georgia 30341

NATIONAL RISK MANAGEMENT RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

NATIONAL CENTER FOR INFECTIOUS DISEASES
DIVISION OF PARASITIC DISEASES
CENTERS FOR DISEASE CONTROL and PREVENTION
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
ATLANTA, GEORGIA 30341

NOTICE

The U.S. Environmental Protection Agency through its Office of Research and Development partially funded and collaborated in the research described here under EPA/NRMRL-HHS/CDC interagency agreement DW 75937984. It has been subjected to the Agencies' peer and administrative review and has been approved for publication as an EPA document.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threatens human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Hugh W. McKinnon, Director
National Risk Management Research Laboratory

ABSTRACT

This document summarizes the application of a particular molecular method to improve detection and differentiation of species and genotypes of *Cryptosporidium* oocysts found in environmental samples. Of particular interest is the method's potential for determining the source animal types of oocysts in water samples. The molecular method is a nested polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) procedure that characterizes the small sub-unit (SSU) ribosomal RNA gene. The method was previously developed for characterizing oocyst DNA from clinical samples. The current project explores the method's applicability to environmental water samples, which have greater diversity of oocyst species and strains, lower concentrations of oocysts, and different interferences than clinical samples. Results include demonstrating that the method is capable of detection and differentiation of at least 10 species and 22 genotypes of *Cryptosporidium*; method sensitivity demonstrated to a single oocyst with laboratory samples; and detection and differentiation of oocysts from oyster gill washings and hemolymph, storm water, surface water, and raw waste water. The method's capability to determine an oocyst's source animal type was demonstrated by identification in environmental water samples of host-adapted *Cryptosporidium* species and genotypes that were consistent with the source animal types (i.e., humans, farm animals, wildlife, and/or pets) inhabiting the sampled watersheds.

TABLE OF CONTENTS

NOTICE	ii
FOREWORD	iii
ABSTRACT	iv
LIST OF FIGURES	vi
ACKNOWLEDGMENTS	vii
INTRODUCTION	1
Cryptosporidiosis and <i>Cryptosporidium</i>	1
Benefits of Identifying Host Range of <i>Cryptosporidium</i> Oocysts in Water	1
Determining the Host Range of Oocysts in Water Samples	2
Developing a Collection of Infection Data for <i>Cryptosporidium</i> -Host Pairs	2
Methods for Detailed Characterization of Oocysts	2
Discovery of Correlations Between Characteristics of Oocysts and Their Host Ranges	3
PROJECT RATIONALE, OBJECTIVES, AND TASKS	4
MATERIALS AND METHODS	4
Materials and Methods for Phylogenetic Analysis of <i>Cryptosporidium</i> Genus Based on SSU rRNA Genes	4
Development Process for SSU rRNA-based Nested PCR-RFLP Method for <i>Cryptosporidium</i> Detection and Differentiation	5
Evaluation of SSU rRNA-based Nested PCR-RFLP Method for <i>Cryptosporidium</i> Detection and Differentiation in Storm Water Samples	6
KEY RESULTS	7
Results of Phylogenetic Analysis of <i>Cryptosporidium</i> Genus Based on SSU rRNA Genes of Five Types of <i>Cryptosporidium</i>	7
Results of Development of SSU rRNA Nested PCR-RFLP Diagnostic Tool	8
Evaluation of the SSU rRNA-based Nested PCR-RFLP Diagnostic Tool	10
Gill Washings and Hemolymph from Oysters	10
Storm Stream Flow Samples	10
Raw Surface Water Samples	11
Raw Wastewater Samples	11
Comparison of PCR Protocols for Species Detection, Differentiation, and Genotyping of <i>Cryptosporidium</i>	11
CONCLUSIONS AND RECOMMENDATIONS	12
Specific Conclusions	12
General Conclusions	14
Recommendations	15
Current problems in molecular detection of <i>Cryptosporidium</i> oocysts	15
Actions needed to enable routine use of molecular tools in water sample analysis	15
REFERENCES	17
APPENDIX 1 – Molecular Tools	19

LIST OF FIGURES

Figure 1. Detection and Diagnosis of <i>Cryptosporidium</i> Parasites by Nested PCR-RFLP	5
Figure 2. Phylogenetic Relationships of <i>Cryptosporidium</i> Parasites to Other Apicomplexans(A) and Each Other(B) (Xiao et al., 1999a)	7
Figure 3. Updated phylogenetic relationship of <i>Cryptosporidium</i> parasites	8
Figure 4. Detection of <i>Cryptosporidium</i> spp. by SSU rRNA-based Nested PCR	8
Figure 5. Differentiation of <i>Cryptosporidium</i> Species and Genotypes by SSU rRNA-based PCR-RFLP	9
Figure 6. Sensitivity of the SSU rRNA-based <i>Cryptosporidium</i> PCR-RFLP Genotyping Technique	10
Figure 7. Differentiation of the <i>Cryptosporidium</i> Parasites in Storm Water Samples by SSU rRNA-based PCR- RFLP.	11

ACKNOWLEDGMENTS

Much of the work described in this report on the SSU rRNA nested PCR-RFLP method was conducted under EPA/NRMRL-HHS/CDC interagency agreement DW 75937984. The work conducted under this interagency agreement built upon method development work previously conducted under EPA/Office of Water-HHS/CDC interagency agreement 75937730, and a substantial amount of collaboration also occurred. Key collaborating researchers include Ronald Fayer, Agriculture Research Service of the U.S. Department of Agriculture; Kerri Alderisio, New York City Department of Environmental Protection; Una Ryan and R.C. Andrew Thompson, Murdoch University, Western Australia; Steve Gradus and Ajaib Singh, City of Milwaukee Public Health Laboratories; Thaddeus K. Graczyk, Johns Hopkins School of Hygiene and Public Health, and Joseph Limor, and Irshad Sulaiman, Centers for Disease Control and Prevention.
